has considered Applicant's arguments, but according to the Examiner, these arguments are not deemed persuasive. The Office Action states that even though Applicant may be the first to demonstrate accurate, real time *in vivo* data demonstrating that GPCRs oligomerize, this concept is allegedly not unobvious over the prior art. According to the Office Action, the prior art teaches that GPCRs are believed to form oligomers (White et al.) and the prior art also teaches that proteins which are believed to form oligomers can be labeled with fluorescent donors and acceptors (Miyawaki et al.). Also stated in the Office Action is that the methods for producing fluorescently labeled fusion proteins of GPCRs would be identical to that for producing fusion proteins of non-GPCRs. Therefore, according to the Office Action, there would have been a reasonable expectation of success for one of ordinary skill in the art at the time of the present invention to have combined the teachings of Miyawaki et al. and White et al. to produce fluorescently labeled GPCR fusion proteins for transfection cell lines. Furthermore, the Office Action states that the artisan would have had a reasonable expectation of success in expressing these fusion proteins on the cell surface in absence of evidence to the contrary. Further stated in the Office Action is that Applicant has allegedly not provided any unexpected results by expressing functional GPCR-fluorescent fusion proteins on the surface of the cell. Therefore, even though Hebert et al. only suggested that real-time *in vivo* FRET analysis of GPCRs would be a logical next step in order to determine *in vivo* oligomerization of FRET receptors, the Office Action concludes that Hebert et al.., in view of White et al. and Miyawaki et al. would render the present invention obvious.

Applicants respectfully point out that although Miyawaki et. al.(*Nature* 388: 882-887, 1997) discloses the use of fluorescence resonance energy transfer (FRET) to detect an intramolecular interaction, this reference is not relevant to the detection of real-time, *in vivo* intermolecular interactions of GPCRs for the following reasons. Detection of a FRET signal requires that all three of the following criteria must be met simultaneously (reviewed in Overton and Blumer, *Methods* 27:324-332, 2002) (Exhibit A): 1) the fluorescence donor (CFP) and acceptor (YFP) must be separated by <100Å ; 2) the donor and the acceptor must be oriented

such that their dipoles overlap; and 3) the mobility of donor and acceptor must be restricted such that criteria 1 and 2 are satisfied with high probability at any given moment, yielding FRET with detectable efficiency. Miyawaki et al. satisfied these three criteria by using a system in which CFP and YFP were covalently attached to a single molecule of calmodulin, whose atomic structure had been solved both in the absence and presence of Ca2+. This structural information made it reasonably likely that FRET could be detected in the presence of Ca2+, but not in its absence, since Ca2+-bound calmodulin was known, prior to the publication of the Miyawaki et al. reference, to form a structure that would bring the appended CFP and YFP domains within sufficient proximity. In contrast, in the absence of Ca2+, calmodulin was known to adopt an open, flexible conformation that would make FRET between appended CFP and YPF domains very unfavorable. These predictions were in fact confirmed by Miyawaki et al. Thus, Miyawaki et al showed that <u>intramolecular</u> FRET could be detected using these fluorophores. However, it does not necessarily follow that there is a reasonable likelihood of success of detecting <u>intermolecular</u> FRET, because any one of the three criteria stated above might not be satisfied when CFP and YPF are attached to separate proteins.

Therefore, an accurate reading of Miyawaki et al. would teach that structural information is required for one to consider that FRET could be used with a reasonable chance of success to detect even an intramolecular interaction. In marked contrast, however, at the time of Applicant's invention, the atomic structure of a GCPR was unavailable, making it very uncertain whether CFP and YPF attached independently to two receptors would satisfy all three criteria needed to detect an intermolecular interaction via FRET. Thus, the expectation of success did not exist at the time of the present invention. Even today, the structure of an active GPCR has not been determined at atomic resolution. Likewise, the structure of a GPCR in a native membrane remains unknown, although very recent studies using powder diffraction and atomic force microscopy of the GPCR rhodopsin in native membranes have confirmed Applicant's *in vivo* studies and have shown the existence of GPCR dimers, at least in the resting (i.e. without light stimulation) state (Fotiadis et al. 2003. Nature 421: 127-8) (Exhibit B).

Furthermore, even if the three criteria listed above are satisfied, detection of an efficient FRET signal requires that the two proteins of interest interact stably and for much of their lifetimes. Prior to Applicant's invention, it was unknown whether GPCRs exist as stable oligomers or whether oligomers form transiently, or only under special circumstances, such as during biogenesis or endocytosis. These considerations likewise made the likelihood of success, based on the prior art, unknown.

Also, FRET measurement with membrane proteins poses a unique problem that had never been addressed prior to Applicant's invention. Since membrane proteins diffuse only in two dimensions, they can collide non-specifically with other membrane proteins and can form complexes with unknown lifetimes. Thus, prior to Applicant's invention, it was unknown whether FRET could occur as a result of such non-specific collisional interactions or whether FRET could occur as a result of specific and stable interactions between proteins in the membrane. Therefore, even if one supposed that GPCR oligomerization could be detected by FRET, controls would be necessary to show that the interaction is in fact due to specific oligomerization and not due to non-specific collisional interaction. The applicant has provided extensive evidence to this effect. Such evidence provides an important distinction relative to Hebert et al. (*Biochem. Cell Biol.* 76: 1-11, 1998), which merely suggested the potential utility of FRET to detect GPCR oligomerization. This reference did not state the many reasons why FRET might not work or why controls would be needed to provide convincing evidence of a specific interaction. Just because Hebert et al. did not mention these problems that would result in failure does not mean that Hebert et al. established reasonable expectation of success. Thus, Hebert et al.'s mere suggestion to use FRET, in the absence of data, does not provide to one of skill in the art a reasonable expectation of success for the real time, *in vivo* detection of GPCR oligomerization via FRET.

Similarly, White et al. (*Nature* 396: 679-682, 1998) does not provide evidence showing that GPCRs are oligomeric *in vivo*. Technically, White et al. provided biochemical evidence (yeast two-hybrid experiments; co-immunoprecipitation experiments) suggesting an interaction between GABA-B(R1) and (R2). These experiments lacked controls, as summarized in various reviews (e.g. Angers et al. *Annu. Rev. Pharmacol. Toxicol.* 2002. 42:409-435; specifically, pg. 410-11) (Exhibit C), needed to indicate whether R1 and R2 association is not an artifact of detergent solubilization or whether the interaction occurs when these receptors are in the native membrane. This is a significant concern, as summarized by Angers et al on p. 410 of the review which states that,"coimmunoprecipitation of membrane receptors requires their solubilization using detergents, and it may be problematic when considering highly hydrophobic proteins such as GPCRs that could form artifactual aggregates upon incomplete solubilization." Indeed, aggregates of GPCRs can be so stable that they resist disruption by denaturing detergents such as sodium dodecyl sulfate (e.g. see Zawarynski et al. *FEBS Lett* 1998 441:383-6) (Exhibit D). Because White et al. did not determine whether GABA-B(R1) and (R2) associate in native membranes and cells, their co-immunoprecipitation data only reveal that a biochemical interaction can occur *in vitro*, which may not be biologically relevant. For this reason, Angers et al. conclude on pg. 411 of their review that, "the general acceptance of GPCR dimerization still awaited the direct demonstration that these complexes existed in living cells." Thus, Angers et al. states explicitly that one skilled in the art would not conclude that GPCR oligomerization would be expected. In fact, it was Applicant's invention that provided this direct demonstration, i.e., the first real time, *in vivo* evidence of G protein-coupled receptor oligomerization in intact cells.

Therefore, it would not have been obvious for one of skill in the art to combine Hebert et al.'s suggestion with the teachings of Miyawaki et al., directed to intramolecular interactions, and the inconclusive teachings of White et al. to arrive at the present invention. Furthermore, the present invention provided the first study that <u>definitively</u> demonstrated *in vivo* G protein-coupled receptor oligomerization in intact cells. As a result of Applicant's invention, these

interactions can now be observed, in real time, and measured accurately. Prior to this work, it was not possible to directly measure the oligomerization of G protein-coupled receptors *in vivo*, nor was it possible to measure the effects of compounds on the oligomerization of G protein-coupled receptors *in vivo*. Therefore, the present invention has provided a better understanding of how G protein-coupled receptors interact *in vivo* as well as a better understanding of how compounds that affect these receptors exert their effects *in vivo*, thus providing a significant advance in the field of receptor biology. Thus, applicants believe this rejection has been overcome and respectfully request its withdrawal.

B. The Office Action states that Claims 5 and 10 remain rejected under 35 U.S.C. § 103(a) and new claims 27-30 are also rejected for reasons of record. The teachings of Miyawaki et al., White et al., and Hebert et al. are discussed above. The Office Action further states that the Gama et al. reference was not cited by the Examiner to show how these truncation mutants can be used in a FRET assay, but that it would have been obvious to produce truncated GPCR for a number of reasons which do not include their use in FRET assay. Also stated in the Office Action is that the claims allegedly do not recite that the truncated GPCRs are in any way involved in the FRET assay, only that the GPCRs are truncated. Therefore, according to the Office Action, regardless of the assay, it would have been obvious to the artisan at the time of the invention to have truncated a GPCR to perform these structure-function studies. Regarding the FRET assay, the Office Action states that it would have been obvious to the artisan to have produced truncation mutants to determine which amino acid residues of the GPCRs were involved in oligomerization. Thus, the Office Action concludes that the teachings of Gama et al. coupled with the teachings of White et al., Miyawaki et al., and Hebert et al., render the present invention obvious.

With regard to the Examiner's statement that the claims do not recite that the truncated GPCRs are in any way involved in the FRET assay, Applicant respectfully points out that claims 5, 10 and 27-30 all depend from claims directed to the use of G protein-coupled receptor fusion

proteins in a FRET assay. Furthermore, it would not have been obvious to produce the truncated GPCRs of Gama et al. for the purposes of the present invention because Gama et al. provides no indication that the interaction between G protein-coupled receptors can be measured and, at the time of Applicant's invention, the atomic structure of a GPCR was unavailable, making it very uncertain whether CFP and YPF attached independently to two receptors would satisfy all three criteria needed to detect an intermolecular interaction via FRET. As stated above, even today, the structure of an active GPCR has not been determined at atomic resolution. Furthermore, even if the three criteria for efficient FRET detection listed above are satisfied, detection of an efficient FRET signal requires that the two proteins of interest interact stably and for much of their lifetimes. Prior to Applicant's invention, it was unknown whether GPCRs exist as stable oligomers or as oligomers that form transiently, or only under special circumstances, such as during biogenesis or endocytosis. These considerations likewise made the likelihood of success, based on the prior art, unknown. Therefore, it would not have been obvious to construct truncated GPCRs in order to measure oligomerization via FRET because it was unclear how GPCRs interacted *in vivo*, much less how truncated GPCRs interacted *in vivo*. It is the present invention that allows direct measurement of the oligomerization of G protein-coupled receptors *in vivo* such that truncated GPCRs can be produced in order to provide valuable information on the structure-function relationship of GPCRs. Thus, applicant believes this rejection has been overcome and respectfully requests its withdrawal.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending claims in this application is believed warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

Payment in the amount of $465.00 (extension of time fee) is to be charged to a credit card and such payment is authorized by the signed, enclosed document entitled Credit Card Payment

Form PTO-2038. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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Gwendolyn D. Spratt — 7-14-03

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